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The Application of the Serum γ-Glutamyl Transpeptidase and the 5'-Nucleotidase Assay in Cancer Patients: A Comparative Study

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This paper deals with comparative sequential studies of serum γ-glutamyl transpeptidase and serum 5'-nucleotidase and serum alanine aminotransferase in cancer patients.
The levels of γ-glutamyl transpeptidase and 5'-nucleotidase, as indicators of metastatic liver, take approximately parallel courses. The levels of γ-glutamyl transpeptidase do not rise earlier than the levels of 5'-nucleotidase but may rise to a greater extent in some cases. Strong increases of γ-glutamyl transpeptidase occur in cases of hepatotoxic liver damage as a consequence of therapy. In a number of cases γ-glutamyl transpeptidase was the only serum enzyme studied that did increase. Increases of γ-glutamyl transpeptidase as a response to therapy might overshadow any response of γ-glutamyl transpeptidase due to the course of malignant process.

In vergleichenden Untersuchungen wird das Verhalten von γ-Glutamyl-Transpeptidase, 5'-Nucleotidase und Alaninaminotransferase im Serum von Krebspatienten mit dem klinischen Verlauf in Beziehung gesetzt. Soweit die Aktivität der γ-Glutamyl-Transpeptidase ein Indikator von Metastasen in der Leber ist, läuft sie parallel mit der Aktivität der 5'-Nucleotidase. Die γ-Glutamyl-Transpeptidase-Aktivität steigt nicht eher als die 5'-Nucleotidase-Aktivität, aber manchmal steigt sie über die der 5'-Nucleotidase hinaus. Starke Erhöhungen von γ-Glutamyl-Transpeptidase treten bei toxischen Leberschäden infolge therapeutischer Verfahren auf. In vielen solchen Fällen war die γ-Glutamyl-Transpeptidase das einzige erhöhte Serumenzym. Diese Reaktion der γ-Glutamyl-Transpeptidase erschwert in hohem Maße die Brauchbarkeit der γ-Glutamyl-Transpeptidase für die klinische Bewertung der Therapie.

Elevation of the serum activity of several enzymes[1]) has been claimed to be an indication of metastatic processes in the liver. Interest was first focussed on transaminases and alkaline phosphatase for this purpose. In more recent years 5'-nucleotidase and γ-glutamyl transpeptidase have also been used for the detection of malignant liver disease. The advantages of 5'-nucleotidase as a diagnostic tool have been summarized in reference (1). γ-Glutamyl transpeptidase has been reported to be increased in patients suffering from metastatic liver (2, 3, 4, 5, 6). This has also been observed with 5'-nucleotidase (1, 7). It has been suggested that γ-glutamyl transpeptidase may replace 5'-nucleotidase for confirming the hepatic origin of raised alkaline phosphatase (8). Preliminary observations however indicated that γ-glutamyl transpeptidase may not have the same reaction pattern as 5'-nucleotidase in malignant liver disease. Therefore an attempt was made to determine the clinical significance of variations of γ-glutamyl transpeptidase in cancer patients.

For this purpose the levels of γ-glutamyl transpeptidase were compared with those of 5'-nucleotidase, whose clinical value has been established by various studies (1, 7, 9).

Attention was paid to the following questions:

a) Are γ-glutamyl transpeptidase activities elevated earlier and in more cases of confirmed metastatic liver than 5'-nucleotidase activities.
b) What is the influence of hepatotoxic therapy on the levels of γ-glutamyl transpeptidase.

Materials and methods

γ-Glutamyl transpeptidase

This enzyme was sometimes assayed according to a manual procedure (10) but in most cases by an automated procedure. Both procedures use γ-glutamyl-*p*-nitroanilide as a substrate. The newly developed flow diagram for the automated procedure is given in figure 1 (1:2 sample/wash ratio). Forty specimens were run per hour. Two heating coils were placed in sequence in order to obtain an incubation time of 13 minutes. The solutions containing the buffer and the substrate were prepared according to *Szasz* (11) and contained 0.1% (v/v) sterox sulution. The fluid stream containing the liberated *p*-nitroaniline was allowed to pass from one part of the system to another through glass tubing. Calibration curves were constructed using pooled sera with different levels of activity as standards. These levels were established by the manual method (ref. 10, with the exception that incubation was performed

[1]) Enzymes
5'-nucleotidase = 5'-Ribonucleotide phosphohydrolase (EC 3.1.3.5.); γ-glutamyl transpeptidase: Not listed in the report of the International Union of Biochemistry (Elsevier, Amsterdam, 1964); alanine aminotransferase = *L*-Alanine: 2-oxo-glutarate aminotransferase (EC 2.6.1.2.); alkaline phosphatase = Orthophosphoric monoester phosphohydrolase (EC 3.1.3.1.).

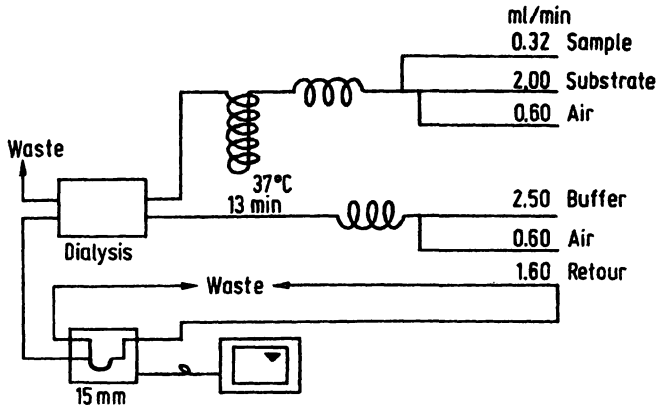

Fig. 1. Flow diagram for the assay of γ-glutamyl transpeptidase.

at 37° C). Use of semilog graph paper simplified the check of linearity of recorded absorbances against activities of the standards during each run. Every tenth specimen was a control of known activity. To prepare control specimens the activity of a pooled serum was measured with the manual procedure. Thereafter aliquots were stored at – 20° C. Standards were also kept frozen. Standards and controls were used only once after thawing. The coefficient of variation of the controls prepared from random sampled sera during one month was 3.8% (mean 85U/l, n = 89).
Comparison of the automated method with the manual method showed a correlation coefficient (r) of r = 0.9917
Normal values: males 6–38 U/l; females 4–24 U/l. The coefficient of variation was found to be 1.5% in the range of slightly elevated values.

5'-Nucleotidase

The procedure as described in ref. 13 was followed.
For comments on the procedure the reader should consult references 7, 14, 15 and 16. Normal values 5.5–11.5 U/l (37° C). The coefficient of variation is 3.5% (see ref. 13) in the range of slightly elevated values.

Alanine aminotransferase

The assay was performed kinetically with an LKB reaction rate analyzer using Boehringer reagent kits (non-optimal assay adapted for automated analysis). The automatic dispenser was set for 100 µl 2-oxoglutarate solution since smaller volumes may give erratic results. The oxoglutarate solution had been diluted so as to obtain the prescribed final concentration during measurement. Normal range till 24 U/l (35° C). The coefficient of variation was found to be 2.0% in the range of slightly elevated values.

Results

γ-Glutamyl transpeptidase and 5'-nucleotidase levels were studied in a group of 33 patients with malignant liver disease. For 31 patients the presence of liver metastases was established by laparotomy. For one patient the probability of metastatic liver was indicated by liver scintigraphy. For the last patient of this group the diagnosis of liver carcinoma was made on the basis of foetoprotein estimation. The primary sites included breast, stomach, rectum, colon and lymph glands. γ-Glutamyl transpeptidase and 5'-nucleotidase values obtained prior to examination by laparotomy etc. are depicted in figure 2. In the majority of cases no other therapeutic measures had been performed. The data show that in 7 cases normal levels of one of the two enzymes

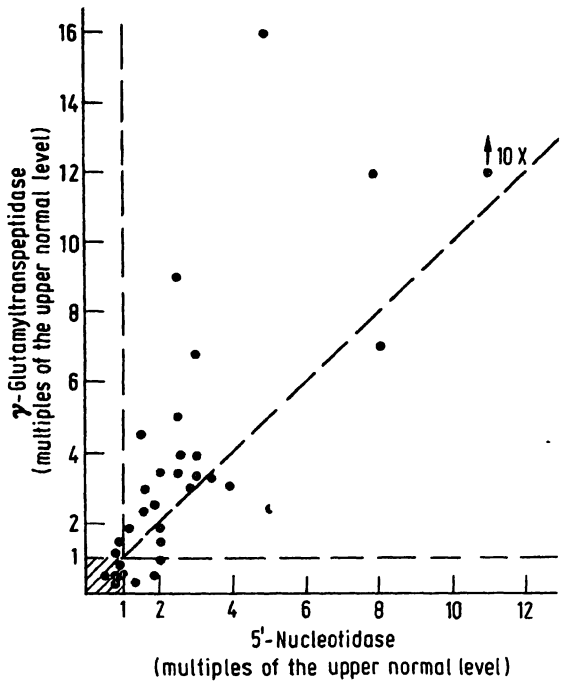

Fig. 2. Comparison of serum 5'-nucleotidase activities and serum γ-glutamyl transpeptidase activities in cases of confirmed liver metastases.

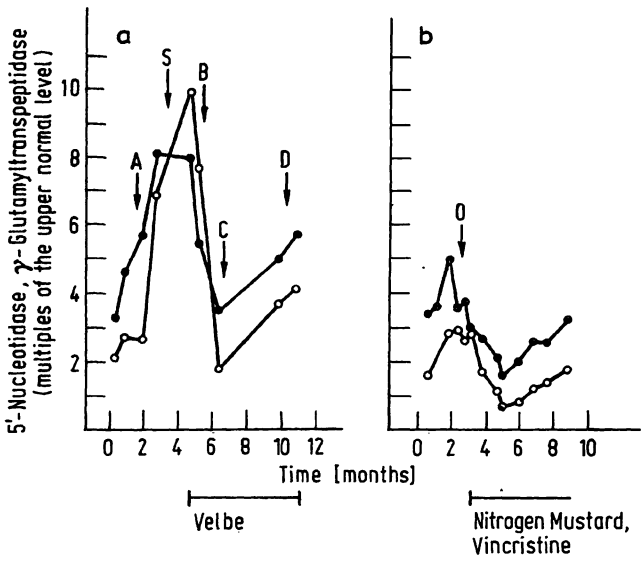

Fig. 3. Variation, with time of γ-glutamyl transpeptidase (o—o) and 5'-nucleotidase (•—•) levels.

were found. In five cases both enzymes had normal levels.
Study of the sequential course of 5'-nucleotidase and γ-glutamyl transpeptidase in a great number of patients revealed no clear evidence that the level of γ-glutamyl transpeptidase rose before that of 5'-nucleotidase. Both enzyme activities take a parallel course. Remarkable deviations were found after the start of hepatotoxic therapy (see below).
In figure 3a, 3b and 4 examples are given of the parallel course of γ-glutamyl transpeptidase and 5'-nucleotidase. Figure 3a and b show changes of γ-glutamyl transpeptidase and 5'-nucleotidase in two patients suffering from

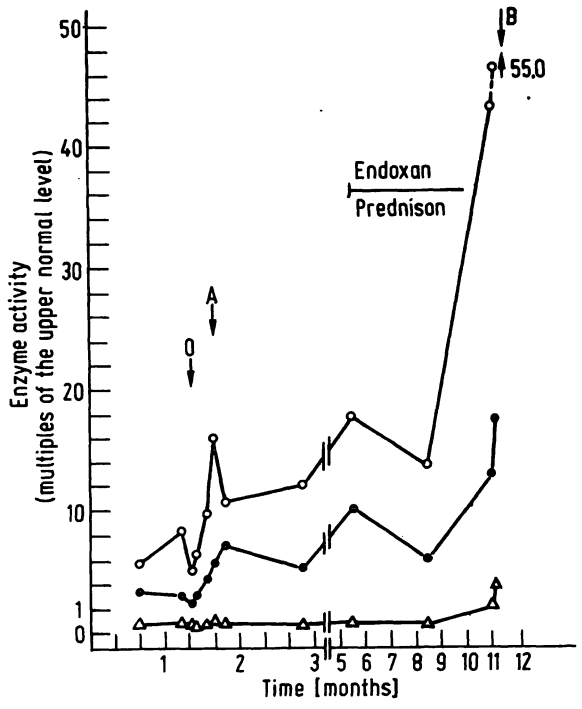

Fig. 4. Variation, with time of γ-glutamyl transpeptidase (o—o), 5'nucleotidase (•—•) and alanine aminotransferase (Δ—Δ) levels.

M. Hodgkin. The liver was found enlarged at the times indicated by arrows A, B and D (fig. 3a). Liver was not palpable at time C. Liver scintigraphy, performed at time S, suggested the existence of liver metastases. At the time indicated by arrow 0 (fig. 3b) laparotomy revealed the existence of metastases in the liver, which was palpable shortly before. Figure 4 may be interesting since the existence of liver metastases have been confirmed at two different times: by laparotomy at time 0 and by autopsy at time B. γ-Glutamyl transpeptidase and 5'-nucleotidase take parallel courses except for the short time following laparotomy (see arrow A).
Additional information is given in table 1.
The data in this table refer to patients in which the existence of metastases in the liver was confirmed by autopsy. The highest value of each serum enzyme shortly before death has been used. When assembling data from laparotomy reports it was found that in 66 cases the liver had been judged to have a normal aspecht. In 11 of these patients γ-glutamyl transpeptidase values were elevated, the 5-nucleotidase values in two of them were normal. It should perhaps be noted that one of these two patients (normal 5'-nucleotidase and elevated γ-glutamyl transpeptidase) was addicted to palphium. In the second patient no clinical signs for the existence of liver metastases became apparent within several months after laporotomy.
The effect of hepatotoxic therapy on the level of γ-glutamyl transpeptidase which was referred to above was studied in the way described in ref. 1. It was found that the start of a given therapy may cause a transient or apparently additional rise in the activity of serum enzymes. The amount of transient increase of serum activity can vary considerably.
Toxic damage resulted in much greater changes in the level of γ-glutamyl transpeptidase than of 5'-nucleotidase. (The latter phenomenom has been described already in ref. 1, 7). In 43 cases in which either one or both serum enzymes showed transient elevations the following observations were made: Increases of γ-glutamyl transpeptidase to an amount less than twice the upper normal level were found only in 10 cases. The greatest increase of γ-glutamyl transpeptidase was twenty times the upper normal level. In 33 cases the transient rise of the 5'-nucleotidase activity was between zero and twice the upper normal level. In 21 cases the rise of 5'-nucleotidase was between zero and the medium normal range. The greatest increase of serum alanine aminotransferase was 23 times the upper normal level. Examples are given in figure 5 and 6. We give for comparative purposes the values of alanine aminotransferase, which is a sensitive monitor for hepatotoxic damage.
Figure 5a concerns a patient suffering from *M. Hodgkin* who was treated with Velbe (at the times indicated by the left and right arrows). Bleiomycin was given a few days after the first Velbe treatment. The liver of the patient was not palpable. Chemotherapy was continued for two months. The serum enzymes were seldom determined and gave elevated γ-glutamyl transpeptidase and normal 5'-nucleotidase values. The patient died shortly after. Autopsy did not show the presence of liver metastases. Figure 5b concerns a patient with tumour in the flour of the mouth, which was treated surgically at the time indicated by the arrow. Note that γ-glutamyl transpeptidase shows the strongest reaction to the therapeutic measures.

Tab. 1. Serum γ-glutamyl transpeptidase, 5'-nucleotidase and alanine aminotransferase in cases of metastatic liver confirmed by autopsy.

	number of cases within					
	normal range	1–2x*)	2–4x*)	4–8x*)	8–16x*)	> 16x*)
5'-nucleotidase	2	3	5	0	5	1
γ-glutamyl transpeptidase	0	3	3	5	1	4
alanine aminotransferase	10	3	3			

*) upper normal level

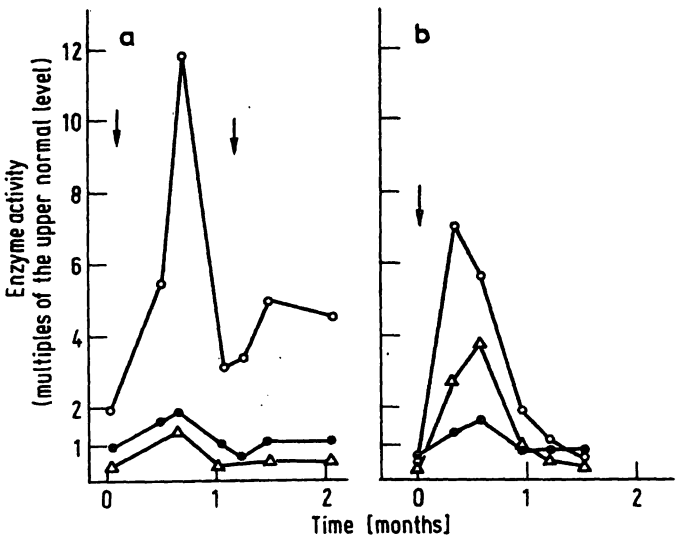

Fig. 5. Variation, with time of γ-glutamyl transpeptidase (o—o), 5'-nucleotidase (•—•) and alanine aminotransferase (Δ—Δ) levels.

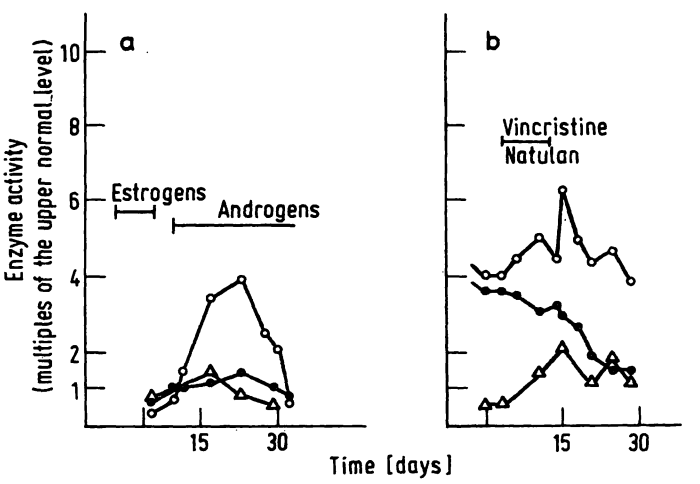

Fig. 6. Variation, with time of γ-glutamyl transpeptidase (o—o), 5'-nucleotidase (•—•) and alanine aminotransferase (Δ—Δ) levels.

Figure 6a shows the variation of serum enzymes in a patient suffering from a breast carcinoma. The liver was normal on palpation during the period covered in the abcissa. Within two months after the androgen therapy the patient's condition improved remarkably. Serum enzymes remained normal.

Figure 6b shows the serum enzyme levels of a patient with *M. Hodgkin* who received a combined chemotherapy. The liver was found to be enlarged before the onset of the therapy but appeared normal three months later. This would indicate that the decrease of 5'-nucleotidase values reflects regression of malignant processes in the liver. Note that the course of γ-glutamyl transpeptidase parallels the course of alanine aminotransferase.

If different courses of therapy are given with short intervals, the sequential responses of serum enzymes

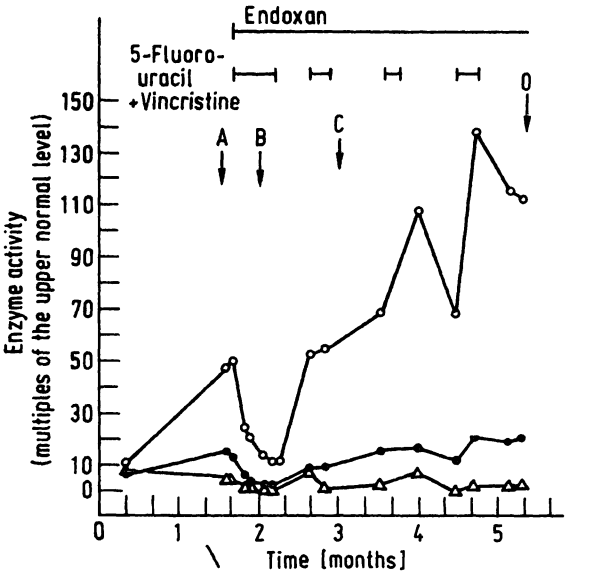

Fig. 7. Variation, with time of γ-glutamyl transpeptidase (o—o), 5'-nucleotidase (•—•) and alanine aminotransferase (Δ—Δ) levels. Patient H. The arrow 0 denotes time of laparotomy.

may overlap. In such cases it is often difficult to establish that a change of enzyme activity reflects an extensive hepatotoxic process. An example is given in figure 7. Patient H (fig. 7) was suffering from breast carcinoma with metastases in bone, liver and lung. The liver was found by palpation to be enlarged at the times indicated by arrows A and C but to be less enlarged at time B. It will be noted that the course of γ-glutamyl transpeptidase shows sharp transient increases after the third and fourth treatments with 5-Fluoro-uracil and Vincristine. The high value of alanine aminotransferase at the start of the curve is due to hepatotoxic damage caused by a preceding methotrexate treatment as has been described earlier (7).

Discussion

Serum 5'-nucleotidase was first reported to rise earlier than alkaline phosphatase in cancer patients by *Van der Slik* et al. In addition 5'-nucleotidase reacts more strongly than does alkaline phosphatase (7, 1). This was confirmed later by *Schwartz* (16). Therefore as a monitor of neoplasm in the liver alkaline phosphatase has lost most of its significance. Alkaline phosphatase is more useful in the follow up of either recalcification of the osteolytic areas (bone repair) or malignant osteoblastic processes. The present study was undertaken to investigate: a) whether γ-glutamyl transpeptidase assays could give additional information to that provided by the assay of other enzymes in current use and b) whether γ-glutamyl transpeptidase could replace one of these enzymes without losing diagnostic information.

Comparison with 5'-nucleotidase, but not with alkaline phosphatase, in view of the advantages cited above seemed to us a logical approach.

The results of this study indicate that γ-glutamyl transpeptidase does not have a sensitivity that exceeds that of 5′-nucleotidase in the sense that it should rise earlier. It may however show stronger elevations than 5′-nucleotidase and for this reason it may replace 5′-nucleotidase in untreated patients. This restriction is necessary in view of the variable changes of γ-glutamyl transpeptidase as a response to hepatotoxic therapy. These changes due to hepatotoxity can be so strong that they may mask any change of γ-glutamyl transpeptidase that reflects the course of a malignant process in the liver. Hepatic injury during therapy may cause elevations of 5′-nucleotidase too, but they are usually small. In this respect advantage can be taken of the fact that alanine aminotransferase reacts quite insensitively to malignant processes of the liver but strongly to hepatotoxic injury. Accordingly the assay of alanine aminotransferase may help to differentiate a rise of 5′-nucleotidase level caused by progression of malignant processes in the liver from one caused by hepatotoxic injury due to the therapy (7, 1). Such a possibility does not exist for γ-glutamyl transpeptidase. It may very well be possible that elevations of γ-glutamyl transpeptidase due to hepatotoxity are not accompanied by an increase of alanine aminotransferase. Consequently, problems in the interpretation of γ-glutamyl transpeptidase values in treated patients will arise especially if γ-glutamyl transpeptidase values change without a concomitant change of alanine aminotransferase. These facts in our opinion argue against a replacement of 5′-nucleotidase by γ-glutamyl transpeptidase in cancer patients under treatment. Because of the greater sensitivity of 5′-nucleotidase than alkaline phosphatase to liver malignancy the situation of an alkaline phosphatase being more elevated than 5′-nucleotidase is a strong indication that bone phosphatase is present in the serum investigated. Thus a combined assay of both alkaline phosphatase and 5′-nucleotidase permits us in some cases to establish the nature of the elevated level of alkaline phosphatase assays without electrophoretic examination (1). Replacement of 5′-nucleotidase by an enzyme like γ-glutamyl transpeptidase that can some times be elevated more strongly, may reduce the number of cases in which the nature of elevated alkaline phosphatase in cancer patients can be established by the assay of a liver-specific enzyme.

Taking the above facts into consideration we incline to the following conclusion. The diagnostic significance of γ-glutamyl transpeptidase in cancer patients lies primarily in the information that is presented when normal values are found. Normal values of γ-glutamyl transpeptidase mean that the liver damage of the patient is minimal during the therapeutic treatment. Normal values of γ-glutamyl transpeptidase, especially when found in consecutive assays combined with other liver tests such as 5′-nucleotidase, indicate with high probability that liver metastases are absent. Following a classification proposed in earlier report (1) γ-glutamyl transpeptidase belongs to group II (serum enzymes which may increase both in cases of liver metastases and in cases of toxic damage of the liver). To this group belong aspartate aminotransferase and glutamate dehydrogenase. In view of its sensitivity γ-glutamyl transpeptidase may rather replace these enzymes than 5′-nucleotidase in the follow up of cancer patients.

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